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DISCLAIMER NOTICE

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?gorov, V. I. and Spitsyn, N. A. (Deceased)

[illegible] Kotorye osobennosti gazoobmena i azotistogo [illegible] v protsesse rosta i razvitiya sibireyaz-vennogo mikroba

[Some peculiarities of the gas and nitrogen exchange in the growth and development of Bacillus anthracis]

[illegible]riya 38(2):45-47. February 1961. [redacted]
[illegible]

(in Russian)

NOT REPRODUCIBLE

Thorough studies of the processes, connected with the via-bility of microorganisms constitute part of the most important tasks of microbiology and biochemistry.

The purpose of ~~our~~ the present work is: a) to study the processes of growth and development of the Bacillus anthracis; b) to estab-lish the quantitative connection between the partial (fractional) [illegible] of oxygen and the intensivity of growth and sporal forma-tion of the microbe and also c) to determine the character of the nitrogen exchange during the cultivation course of the microbe in [illegible]ficial nutrient medium.

The method. The microbial culture was grown in enzyme (fer-[illegible]) hydrolysate according to Hottinger's, in dilution 1:9, con-taining 110 to 120 mg% general nitrogen and 30 to 40 mg% amino acids; pH of the medium equalled 7.2; using the method of deep [illegible]ation with the recirculation of the aerating air and simultane-ously ([illegible]rallel) under conditions of an aeration "upon exhaust".

Figure 1 shows the laboratory scheme of the installation for the cultivation of the microbial culture. The suspension of spores of the vaccinal strain of the microbe (STI strain), at the ratio of 10 millions per 1 ml, was used as seeding material. The temperature, maintained during the cultivation, was 34°. The speed of the air exchange, occurred in the cultivator at the ratio of F liter air per 1 liter medium in one minute.

During the growing of the microbe in the culture under recirculation conditions of the gas phase at that stage of the process, when the concentration of the vegetative forms of the culture has reached the size of the order 0.1 milliard cells per 1 ml, then the cultivator was scrubbed (washed) with the aid of pure oxygen until the analysis showed the content of oxygen (90 to 95%) in the gas phase; thereafter, the cultivation process was continued. The losses of oxygen, which occurred during the mentioned process, were compensated (in the cultivator) by the addition of pure gas from the gasometer. Ammonia gas and carbon dioxide, isolated during the process, were caught in the scrub flasks, containing solutions of the respective absorbents.

Figure 1. Laboratory installation for growing cultures of microbes under conditions of recirculating the aerative air.

1. Cultivator; 2. foam catcher; 3 and 4. scrubber flasks (washers); 5. circulation pump; 6. gasometer; 7. sample catcher; 8, 9, 10. filters.

The oxygen amount, used in the process of cultivation, as well as that of ammonia and carbonic acid, produced during the course of the process, were established during each experiment. After centrifugation, the content of the general and amino nitrogen in the liquid phase as well as that of nitrogen in the microbial cells (bacilli and spores) was established in samples, which were taken periodically from the cultivato.. The beginning and end of sporulation, also the relative content of spores in the culture was controlled by means of microscoping the smears. The concentration of the microbial cells in the culture was determined by the method of seeding on agar.

The growth and sporulation of the Bacillus anthracis occurs considerably more intensively during its cultivation in the atmosphere of oxygen, than in cases, when the culture is aerated by

means of the air atmosphere (fig. 2).

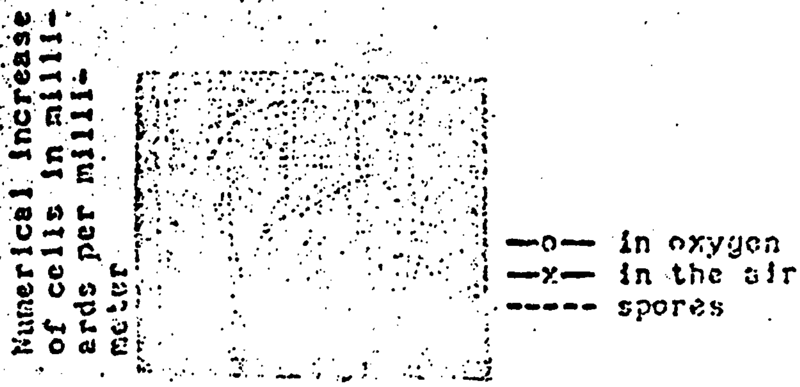

Figure 2. Dynamics of the development of the microbial culture, grown in oxygen atmosphere and in air atmosphere.

Data, concerning the norms of oxygen consumption during the cultivation, evidence the decrease of the energetic standard of the process during the aeration of the developing culture with air (fig. 3).

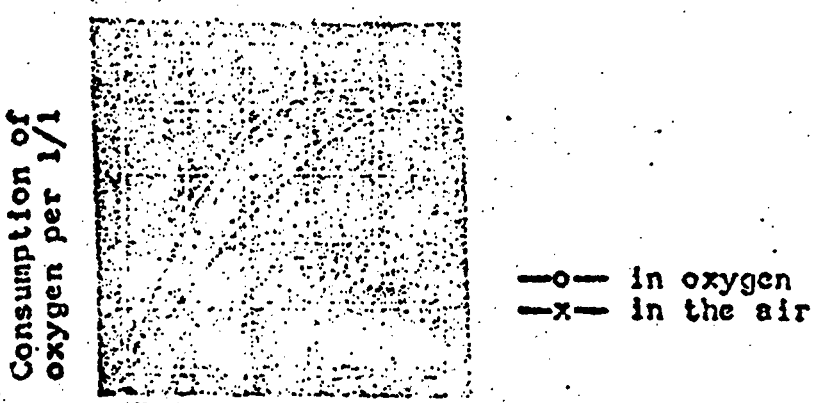

Figure 3. Dynamics of the consumption of oxygen during the development of the culture in the oxygen atmosphere and in the air atmosphere.

It must be emphasized that the intensity of the consumption of

oxygen by the developing culture starts to decrease at the instance, when the process of microorganism (bacilli) sporulation begins; it continues to decrease until accumulation of spores in the culture occurs, consequently, it is obvious, that mainly oxygen is needed during the vegetation period; this requirement decreases considerably (almost completely) during the state of physiological maturing.

The quantitative data, indicating the accumulation of carbon dioxide and ammonia above the growing culture during the gas phase, prove that the beginning of the transformation of the character of the gas exchange occurs during the transition of the culture from one state to another, qualitatively a different state from the preceding, namely into the state of sporulation (table 1).

Table 1

Changes in the character of the gas exchange

Consumption and liberation of gases (mg%)	Existence of the culture (in hours)				
	12	24	36	48	60
Utilized O_2	68	226	360	460	472
Liberated CO_2	17	40	75	112	160
Liberated NH_3	0.15	0.50	1.7	3.7	8.4

Remark: Beginning of sporulation: at the 48-th hour.

According to the table, it is obvious that though the consumption of oxygen practically stops at the stage of sporulation, however, the production of carbon dioxide still continues, whereas the

liberation of ammonia reaches its highest norms during the last stages of the process.

Taking into consideration the fact, that a quite large amount of ammonia remains in the fluid substrate in a fixed state, one may assume that accumulation of [inorganic] nitrogen (mineral form) in the nutrient medium is the connecting factor between this stage [illegible] of sporulation (fig. 4).

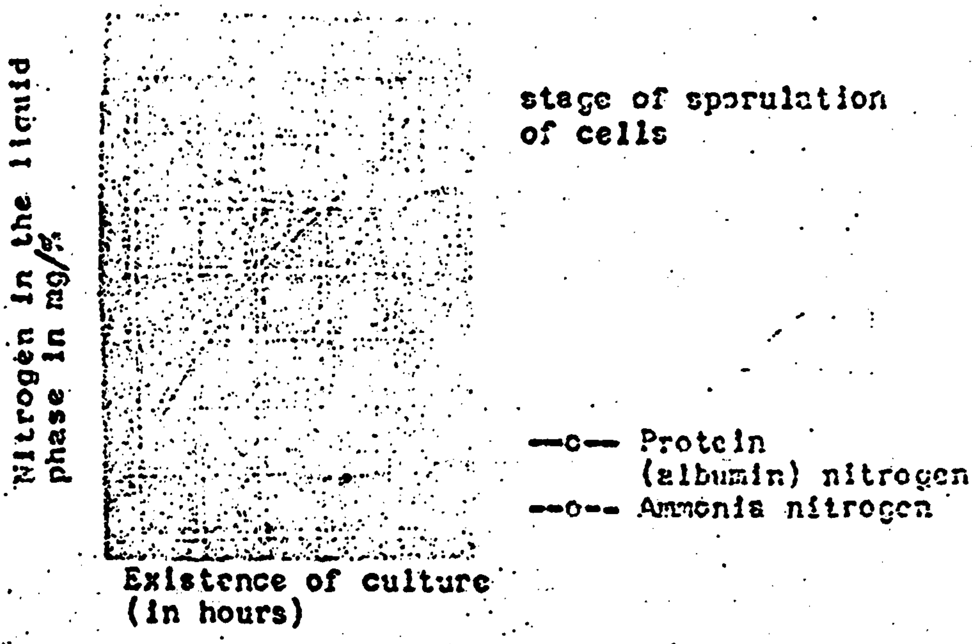

Figure 4. Utilization of protein (albumin) nitrogen and accumulation of ammonia nitrogen in the process of growing the microbial culture.

The mineralization of the protein (albumin) substrate precedes the sporulation [Begin p. 47] of the vegetative cells. Sporulation begins at the point, when in the medium the ratio of the protein and mineral forms of nitrogen changes and the predominance of the mineral forms becomes obvious. During sporulation, the utilization

of protein nitrogen is completely discontinued. The nitrogen balance during the course of cultivation is shown in table 2.

Table 2

Balance of nitrogen during cultivation

Transformed protein nitrogen (in %)		Increase of ammonia nitrogen (%)		Content of nitrogen in cells, resp. in spores (%)
Total	Including amines	In the liquid phase	In the gas phase	
[illegible]	15	40	2	8/7

Based on the data, shown in table 2, it is evident that during the stage of vegetative multiplication Bacillus anthracis utilizes comparatively a small amount of amino-acids, and that it develops predominantly on the account of the destruction of more complicated protein complexes. This proves again the high proteolytic activities of Bacillus anthracis. It is worth noting, that in respect to the content of nitrogen (and ash) the analysis of spores and vegetative cells did not show an essential difference between them.

In the light of the detected peculiarities of nitrogen nutrition and gas exchange in the anthrax microbe, there is reason to expect a more definite explanation of the conditions needed for the formation of the spores. Ordinarily, these conditions are known as "starvation conditions". If we would still use the mentioned terminology, then the expression "starvation conditions" would mean nothing else but mineralization of the medium of the microbe's habitat, that means neither exhaustion of the nutritive medium, nor

the total using-up of protein substances, necessary for vegetative growth. Evidently, the enzyme system, the activities of which are selectively directed toward the protein metabolism, is arrested when the mineralization of the protein medium (the transparency of the cell protoplasm increases under the mentioned conditions) reaches a certain point.

The poorer the medium of protein substances was, the faster the relative equilibrium occurred in the medium between the protein and mineral forms of the nitrogen; it was followed by the predominance of the latter. The mentioned balance of the two nitrogen forms cannot be achieved by growing the microbial culture on a medium rich with protein substances. As well-known, in the latter case, sporulation is completely absent, because not finding the conditions, necessary for the transition into the next state, the cells are taking involutionary forms and start to degenerate.

The phenomenon, mentioned above, could be eliminated by changing artificially the nitrogen compound of the liquid medium, thus influencing the process toward the gelatin direction.

The cultivation of microbes in an atmosphere, enriched with oxygen, opens new opportunities for the intensification of the processes of growth and sporulation.

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